10/541058
14\ pets JC20 Rec'd PCT/PTO 29 JUN 2005

DERIVATIVES OF 4-NITRO-2-(4'-METHOXY-PHENOXY)-METHANESULFONYL ANILINE AND THEIR PHARMACEUTICAL USES

Technical Field

The present invention relates to derivatives of 4-nitro-2-(4'methoxy-phenoxy)-methanesulfonyl aniline and their pharmaceutical uses, especially in the preparation of anti-inflammatory and analgesic drugs.

Background Art

Non-steroid anti-inflammatory drugs (NSAIDs) possess anti-inflammatory and analgesic effects, and have been extensively used clinically in treatment of various inflammations (such as osteoarthritis, inflammation of respiratory tract), tumor, thromboangiitis, postoperative pain, dysmenorrhea, diseases of the ear, nose and throat, and post-traumatic pain, inflammation, fever and other symptoms, in particular, they are more commonly used in arthritis. The role of prostaglandin in the induction of inflammation and the enzyme for its synthesis, cyclooxygenase, were discovered in the 1970s. Of course, the beneficial actions of prostaglandin have also been confirmed, such as providing protection for cells of the gastrointestinal tract, maintaining normal renal function, facilitating platelet aggregation, and the like. It was discovered later that cyclooxygenases can be classified into 2 groups, i.e. COX_1 and COX_2. COX_2 is mostly located in inflammation sites. Therefore, COX_2 inhibitors can affect the generation of prostaglandin in said location, resulting in anti-inflammatory and analgesic effects, with greatly reduced side-effects such as gastrointestinal ulcer and bleeding. In addition, they can inhibit and clear harmful superoxide radicals, hydrogen peroxide radicals, inhibit the synthesis of platelet activating factor, the release of tumor necrosis factor-α, the proteolytic enzymes, and the release of histamine, thus contributing to the anti-inflammatory and analgesic effects. Their anti-inflammatory, analgesic

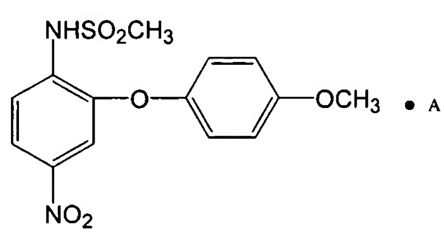
and antipyretic effects have been demonstrated in various experimental models. Therefore, NSAIDs, which belong to COX_2 inhibitors, are commonly used in clinical context. Among them, nimesulide, a selective COX_2 inhibitor, is extensively used for its remarkable therapeutic effects and low toxic side-effects. However, in recent years, there have been a number of reports worldwide of severe liver damage associated with administration of nimesulide. Therefore, there is still a need for developing new COX_2 inhibitors with even better therapeutic effects and lower side-effects.

Disclosure of the invention

The purpose of the present invention is to search and develop a COX_2 inhibitor with better therapeutic effects and lower side-effects.

The inventors, after extensive studies, have surprisingly found that 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline and its derivatives exhibit superior anti-inflammatory and analgesic effects, as compared with nimesulide, a known representative compound of this kind.

Therefore, in a first aspect, the present invention relates to 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline of formula (1)



Formula (1)

or pharmaceutically acceptable salts, solvates or hydrates thereof, where

A is present or absent, and represents a pharmaceutically acceptable inorganic or organic base, or a basic amino acid.

In another aspect, the present invention relates to a mixture of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline, with povidone, phospholipid or cyclodextrin.

The present invention further relates to a pharmaceutical composition for the prevention or treatment of various inflammation, pain, and the like, comprising 4-nitro-2-[(4'-methoxy)-phenoxy]-methanesulfonyl aniline, or a pharmaceutically acceptable salt, solvate, hydrate thereof, or a mixture thereof with povidone, phospholipid, or cyclodextrin, and a pharmaceutically acceptable carrier.

The present invention, in a further aspect, relates to the use of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline, or a pharmaceutically acceptable salt, solvate, hydrate thereof, or a mixture thereof with povidone, phospholipid, or cyclodextrin, in the preparation of non-steroid anti-inflammatory analgesic drugs.

The present invention also relates to a method for fighting against inflammation and pain, comprising administering to a patient in need thereof 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline, or a pharmaceutically acceptable salt thereof, or a mixture thereof with povidone, phospholipid, or cyclodextrin.

According to the present invention, a pharmaceutically acceptable salt of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline, as used herein, refers to a salt formed by 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline with a pharmaceutically acceptable inorganic or organic base, or a basic amino acid, for example, a salt with trans-4-methyl-cyclohexylamine, trans-4-tert-butyl-cyclohexylamine, arginine, or lysine.

According to the present invention, the mixture of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline with povidone, phospholipid, or cyclodextrin in the present invention can be in various forms. For example, a mixture of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline and povidone can be in the form of an associate of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline with povidone; a mixture of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline and phospolipid can be in the

form of a complex of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline with phospholipid; and a mixture of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline with cyclodextrin can be in the form of an inclusion of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline and cyclodextrin.

According to the present invention, the povidone used in the present invention can be various kinds of commercially available povidones, such as povidone K30 (PVP K30, for short); and the cyclodextrin used in the present invention can be various kinds of commercially available cyclodextrins, such as α-, β-orγ- cyclodextrin.

According to the present invention, the pharmaceutically acceptable carrier, as used herein, refers to those substances that have no adverse effects on the active ingredient of the medicament, such as excipients, additives, disintegrants, adhesives, or the like well known in the art.

According to the present invention, the inflammation and pain, as used herein, include, but not limited to: osteoarthritis, respiratory tract inflammation, tumor, thromboangiitis, postoperative pain, dysmenorrhea, inflammation of the ear, nose, and throat, post-traumatic pain, fever, and the like.

According to the present invention, 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline can be administered alone or in the form of a pharmaceutical composition. The pharmaceutical composition of the present invention can be administered orally, parenterally, or topically, and the dosage form can be tablet, capsule, drop, injection, suppository, patch, ointment, and other formulations for oral administration, injection and topical application.

According to the present invention, the pharmaceutical composition can be prepared by well-known methods in the art, e.g. by mixing the compound of formula (1) with other drugs or with a pharmaceutically acceptable carrier.

According to the present invention, the mixture or pharmaceutically acceptable salt of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline

can be prepared by mixing 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline (S_6) with povidone-serial compounds, phospholipid, cyclodextrin, trans-4-methyl- cyclohexylamine, trans-tert-butyl-cyclohexylamine, or a basic amino acid.

Description of figures

Fig 1 shows the X-ray diffraction pattern of S_6.

Fig 2 shows the X-ray diffraction pattern of S_6 PM1.

Fig 3 shows the X-ray diffraction pattern of S_6 PM3.

Fig 4 shows the X-ray diffraction pattern of S_6 PM5.

Fig 5 shows the X-ray diffraction pattern of S_6 K30-1.

Fig 6 shows the X-ray diffraction pattern of S_6 K30-3.

Fig 7. shows the X-ray diffraction pattern of S_6 K30-5.

Fig 8 is the DSC graph of S_6.

Fig 9 is the DSC graph of S_6 K30-1.

Fig 10 is the DSC graph of S_6 K30-3.

Fig 11is the DSC graph of S_6 K30-5.

Fig 12 is the DSC graph of S_6 PM1.

Fig 13 is the DSC graph of S_6 PM3.

Fig 14 is the DSC graph of S_6 PM5.

Mode of carrying out the invention

The following examples will illustrate the present invention in detail, but in no ways limiting the claims of the present invention.

Preparation Example 1. Preparation of 2-(4'-methoxy-phenoxy)-methanesulfonyl aniline

1. Preparation of 2-(4'-methoxy-phenoxy)-nitrobenzene(A)

16g (0.129 mol) of para-methoxyphenol and 8g (0.143 mol) of potassium hydroxide were weighed and placed into a 50ml three-necked flask

equipped with magnetic stirrer, thermometer and condensation tube. The reaction was heated to dissolve the solid. After brief cooling, about 1g of active copper and 15.8g (0.1mol) of 1-chloro-2-nitrobenzene were added, and heating was continued at reflux for 1 hour, keeping the internal temperature around 150°C. After the completion of the reaction, at 80°C, the reaction was poured into 300ml of 3% NaOH and left overnight. Yellow crystals separated out and were subjected to suction filtration, washed with distilled water to neutrality, and dried at room temperature, to give 17.4g of crude product.

17.4g of the above crude product was dissolved under heating in 200ml absolute ethanol. 6g of activated carbon was added and the reaction was refluxed for 5-10 min for decoloration. The reaction was filtered while hot, and the filtrate was cooled thoroughly for the product to separate out. After suction filtration and drying, 16.6g of (A) as a pale yellow solid was obtained. m.p.: 75-77. Thin layer chromatography: (developer: ethyl acetate/ petroleum ether 1:5).

2. Preparation of 2-(4'-methoxy-phenoxy) -aniline(B)

(A) was dissolved in q.s.. ethyl acetate, and subjected to catalytic hydrogenation in the presence of Raney Ni, at 40°C, under 10kg pressure. After the completion of the reaction, the catalyst in the hydrogenation reaction was filtered off. The filtrate was concentrated under reduced pressure, to obtain a sticky product. The latter was dissolved in 10% HCl solution, adjusted to pH 4-5, filtered, and the filtrate was alkalified to pH 9-10 with 25% NaOH solution under cooling and stirring. The reaction was allowed to stand, and the organic phase separated. The aqueous phase was extracted twice with ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate, filtered, and the filtrate concentrated, to give 14g of (B) as a sticky product.

3. Preparation of 2-(4'-methoxy-phenoxy)-methanesulfonyl aniline.

10g of (B) and 10ml anhydrous pyridine were added into a 100ml four-necked flask equipped with stirrer, thermometer, condensation tube and dropping funnel, heated to an internal temperature of 75°C. 6g of methanesulfonyl chloride was added slowly, keeping the internal temperature at 85-90°C, and the reaction was continued for one hour. After the completion, the reaction was poured into 18% aquesous HCl, filtered, and the filtrate was extracted three times with ethyl acetate, washed with water to neutrality. After drying over anhydrous magnesium sulfate overnight, the reaction was filtered, and ethyl acetate was evaporated off under reduced pressure. The reaction was left under cooling to give the crude 2-(4'-methoxy-phenoxy)-methanesulfonyl aniline.

The above crude product was dissolved under heating in 135ml of 95% ethanol. 4g of activated carbon was added and the mixture was refluxed for 5-10 min for decoloration, followed by filtration while hot, and the filtrate was cooled thoroughly for the product to separate out. Suction filtration followed by drying gave 9g of 2-(4'-methoxy-phenoxy)- methanesulfonyl aniline, as white crystals. m.p. 81-83°C. Thin-layer chromatography: (developer: ethyl acetate/petroleum ether 1:5). E.A.: $C_{14}H_{15}NO_4S$, MW: 293.39

	C (%)	H (%)	N (%)
Calculated	57.32	5.15	4.77
Found	57.13	5.04	4.70

Example 1. Preparation of 4-nitro-2-[(4'-methoxy)-phenoxy]-methanesulfonyl aniline and its derivatives.

I. Preparation of 4-nitro-2-[(4'-methoxy)-phenoxy]-methanesulfonyl aniline (S_6) and its mixtures or associates with PVP_{K30} (S_6 K30-1, S_6 K30-3, S_6 K30-5)

3.6g (0.01227 mole) of 2-(4'-methoxy-phenoxy)-methanesulfonyl

aniline, 30ml of glacial acetic acid and 12ml of acetic anhydride were placed in a 100ml three-necked flask equipped with stirrer, thermometer, and dropping funnel, stirred, and heated to a temperature between. 85 and 90°C. 3g of nitric acid was slowly added dropwise. After standing under cooling, yellow crystals separated out, and were subjected to column chromatography on silica gel, eluting with dichloromethane. The fraction with R_f= 0.796 was collected. The combined eluates were concentrated under reduced pressure, to give a pale yellow solid, which was recrystallized from ethanol to obtain 3.5g (84.3% yield) of (S_6) as pale yellow crystals. M.p: 130-132°C. Molecular formula: $C_{14}H_{14}N_2O_6S$. Molecular weight: 338.33.

Elemental Analysis:

calculated	C	49.70%	H	4.70%	N	8.28%
found	C	49.62%	H	4.11%	N	8.07%

A solution of 100mg S_6 in absolute ethanol was added under stirring to a solution of 100mg, 300mg, or 500mg PVP_{K30} in ethanol, respectively, and a yellow clear solution was obtained. Then the solution was concentrated under reduced pressure at 60°C to obtain a yellow oily product, which was cooled with ice and triturated with diethyl ether, to give a yellow solid. After filtering and drying under vacuum, a yellow powder was obtained, designated as S_6K30-1, S_6K30-3 and S_6K30-5, respectively.

100mg solid powder of S_6 was mixed with 100mg, 300mg or 500mg solid powder of PVP_{K30}, and triturated to obtain a homogenous mixture, designated as S_6PM1, S_6PM3 and S_6PM5, respectively.

Results of differential scanning calorimetric analysis (See Fig 1. DSC curve)

From the DSC curve it can be seen that there is a sharp endothermic peak at 132.03°C for S_6 (melting point) . The endothermic peak of PVP_{K30} is at 86.55°C. For the physical mixtures of S_6 with PVP_{K30}, S_6PM1, S_6PM3 and

S_6PM5, there are endothermic peaks of S_6 at 127.84°C, 127.1°C and 129.15°C, respectively, whereas in the DSC curves of $S_6K_{30\text{-}1}$ and $S_6K_{30\text{-}3}$, the endothermic peak of S_6 was also present, but with a shift to the left to different extents. In the DSC curve of $S_6K_{30\text{-}5}$, the endothermic peak of S_6 completely disappeared, indicating that the S_6 crystal is completely inhibited by $PVPK_{30}$, resulting in forming an amorphous substance.

The results of X-powder diffraction (Fig 2) showed that for S6, there were a series of characteristic diffraction peaks at 4.7°-39.1°, whereas for PVP_{K30}, there is no characteristic peak between 4.7°-39.1°. For S_6PM1, S_6PM3, S_6PM5, $S_6K30\text{-}1$ and $S_6K30\text{-}3$, diffraction peaks of S_6 crystal were still present, but the peak heights were greatly reduced. Only in the X-powder diffraction pattern of $S_6K30\text{-}5$, the diffraction peaks of S_6 crystal were completely absent, which was consistent with the results of DSC analysis.

The above results indicate that S_6 can associate with PVP_{K30} via hydrogen bond to form a new amorphous substance.

II. Preparation of 4-nitro-2-[(4'-methoxy)-phenoxy]-methanesulfonyl aniline, trans-4-methyl-cyclohexylamine salt (S_9).

6.8g of 4-nitro-2-(4'-methoxy-phenoxy)-methanesulfonyl aniline was dissolved in 40ml of dichloromethane, and 3.2g of trans-4-methyl-cyclohexylamine was added dropwise under stirring at room temperature. The reaction was heated at reflux for 1 hour, and yellow needle crystals separated out. The reaction was left to cool, and the crystals were collected by filtration, and washed with ethanol for 3 times. After drying under vacuum at 40°C, 6.7g of 4-nitro-2-(4'-methoxy-phenoxy)- methanesulfonyl aniline trans-4-methyl- cyclohexylamine salt was obtained. M.p.: 159-161°C.

Molecular formula: $C_{21}H_{29}N_3O_6S$.

Molecular weight: 451.53

Elemental Analysis:

calculated	C	55.86%	H	6.47%	N	9.31%
found	C	55.88%	H	6.40%	N	9.12%

Example 2: Biological evaluation of 4-nitro-2-[(4'-methoxy)-phenoxy] -methanesulfonyl aniline derivatives

Anti-inflammatory and analgesic effects of 4-nitro-2-[(4'-methoxy)-phenoxy] -methanesulfonyl aniline derivatives (S_6 and S_9) in mice

In the following biological experiments, the analgesic effects of the compounds on acetate-induced body twisting in mice and their effects against carrageenin-induced paw inflammation in mice were observed, and their therapeutic effects were evaluated, using nimesulide as the positive control. The results are listed in Table 1-5.

Table 1

Effects of orally administered S_6 and S_9 against paw inflammation induced by carrageenin in mice

Drug	Dose (mM/Kg)	Number of animals	Paw Swelling mm (M±SD) at 3 hours
Control (DMSO)	0.05ml/20g	16	3.56±0.81
Nimesulide	0.243	8	2.10±1.39**
S_6	0.243	8	0.58±0.67**
S_9	0.243	8	1.18±0.65**

** P<0.01 vs. DMSO control

Table 2

Effects of orally administered S_6 and S_6K30-5 against paw inflammation induced by carrageenin in mice

Drug	Dose (mM/Kg)	Number of animals	Paw Swelling mm (M±SD) at 6 hours
Control (DMSO)	0.05ml/20g	12	4.20±0.65
Nimesulide	0.485	12	2.58±1.20**
S_6	0.485	12	1.91±0.66**
S_6K30-5	0.485	12	1.50±0.95**

**P<0.01 vs. DMSO control

Table 3

Effects of orally administered S_6 against acetate-induced body twisting in mice

Drug	Dose (mM/Kg)	Number of animals	No. of body-twistings (M±SD) at 1 hour
Control (DMSO	0.05ml/20g	10	48.94±11.9
Nimesulide	0.97	10	14.8±11.7**
S_6	0.485	5	11.4±5.3**
	0.97	10	10.8±11.4**

**P<0.01 vs. DMSO control

Table 4

Effects of orally administered S_9 against acetate-induced body twisting in mice

Drug	Dose (mM/Kg)	Number of animals	No. of body-twisting (M±SD) at 3 hours
Control (DMSO)	0.05ml/20g	12	43.4±12.4
Nimesulide	0.485	8	11.1±9.7**
S_9	0.485	8	6.7±6.2**

*P<0.05, **P<0.01 vs. DMSO control

Table 5

Effects of orally administrated S6K30-5 against acetate-induced body twisting in mice

Drug	Dose (mg/Kg)	Number of animals	No. of body-twisting (M±SD) at 6 hours
Control (DMSO)	0.05ml/20g	12	29.84±11.99
Nimesulide	0.97	12	21.00±19.09
S6K30-5	0.97	12	4.81±5.34**

**P<0.01; *P<0.05 vs. DMSO control

The experimental results of paw inflammation induced by carrageenin in mice show that the anti-inflammatory effects of S_6, S_9 and S_6K30-5 are all significantly stronger than that of nimesulide, wherein, the anti-inflammatory effects of S_9 (0.243 mM/kg) at 3 hours are 2 times stronger than nimesulide (0.243mM/kg), S_6 (0.243mM/kg) about 4 times stronger than nimesulide (0.243mM/kg). The anti-inflammatory effects of S_6K30-5 (0.485mM/kg) at 6

hours are about 2 times as strong as nimesulide.

The experimental results of analgesic effects on body-twisting induced by acetic acid in mice show that the analgesic effects of S_6, S_9 and S_6K30-5 are all significantly stronger than nimesulide, wherein, the analgesic effects of S_6 and S_9 (0.485mM/kg) are about 2 times as strong as nimesulide (0.97mM/kg), and the analgesic effects of S_6 K30-5 at 6 hours are about 4-5 times stronger than nimesulide, with a longer duration as well.

The results of acute oral toxicity of 4-nitro-2-[(4'-methoxy)-phenoxy]-methanesulfonyl aniline derivatives (S_6, S_6K30-5 and S_9) in mice (Table 6)

Table 6

Comparison of acute toxicity of S_6 , S_6K30-5 and S_9 orally administered in mice

Drug	LD_{50} value (95% C.L.) mg/kg
Nimesulide	643.9
S_6	2355.5
S_6K30-5	6711.6**
S_9	1223.3

**corresponds to 1118.6 mg/kg S_6

The experimental results of acute toxicity of S_6, S_6K30-5 and S_9 orally administered in mice show that S_6 has the lowest toxicity (LD_{50}=2355.5mg/kg), which is about 4 times lower than that of nimesulide. The toxicity of S_9 and S_6K30-5 is about 2 times lower than that of nimesulide. Moreover, no evident pathological changes were found in the internal organs of survived animals upon anatomic examination.

The experimental results of anti-inflammatory and analgesic effects, and of toxicity show that 4-nitro-2-[(4'-methoxy)-phenoxy]-methanesulfonyl aniline and its derivatives exhibit anti-inflammatory and analgesic effects that are

significantly stronger than those of nimesulide, with a long duration of action and low toxicity.

According to the present invention, the above compounds can be formulated into enteral or parenteral preparations by known methods, such as tablets, capsules, granules, injections, suppository, drops, liniments, for oral, topical administration, injection, or the like.

The above experimental results indicate that 4-nitro-2-[(4'methoxy)-phenoxy]-methanesulfonyl aniline (S_6) and its derivatives (such as S_6K30-5 and S_9) possess anti-inflammatory and analgesic effects that are significantly stronger than nimesulide, while their toxicity is significantly lower, and the duration of action is longer.